Figure 1 Kinetic constants of deoxynucleoside kinases

		dThd	dCyd	dAdo	dGuo	ATP
Dm-dNK	k_c (1/s)	15	15	17,8	18,9	15
	K_m (μM)	0,9	1	109	650	1
	k_c/K_m $M^{-1}s^{-1}$	1,7 x 10^{7}	1,5 x 10^{7}	1,6 x 10^{5}	2,9 x 10^{4}	1,5 x 10^{7}
hTK1 (a)	k_c (1/s)	4,2				4,2
	K_m (μM)	0,5				140
	k_c/K_m $M^{-1}s^{-1}$	8,3 x 10^{6}				3 x x 10^{4}
hTK2 (b)	k_c (1/s)	0,3	0,4			,0,3
	K_m (μM)	16	16			2
	k_c/K_m $M^{-1}s^{-1}$	1,9 x 10^{4}	2,5 x 10^{4}			1,5 x 10^{5}
hdCK (c)	k_c (1/s)		0,073	0,31	0,4	0,073
	K_m (μM)		1	120	150	10
	k_c/K_m $M^{-1}s^{-1}$		7,3 x 10^{4}	2,6 x 10^{3}	2,7 x 10^{3}	7,3 x 10^{3}
hdGK (d)	k_c (1/s)			1,35 x 10^{-3}	2,1 x 10^{-3}	
	K_m (μM)			60	7,6	
	k_c/K_m $M^{-1}s^{-1}$			2,3 x 10^{1}	2,8 x 10^{2}	
E. Coli TK (e)	k_c (1/s)	1,6				1,6
	K_m (μM)	9				500
	k_c/K_m $M^{-1}s^{-1}$	1,8 x 10^{5}				3,2 x 10^{3}
HSV TK (f)	k_c (1/s)	0,21				0,21
	K_m (μM)	0,6				32
	k_c/K_m $M^{-1}s^{-1}$	3,8 x 10^{5}				6,6 x 10^{3}
Lactobac. dAK/dCK (g) or dGK	k_c (1/s)		0,91	0,05	0,7	0,91
	K_m (μM)		4,5	8,4	7,8	1000
	k_c/K_m $M^{-1}s^{-1}$		2 x 10^{5}	6 x 10^{3}	8,9 x 10^{4}	9,1 x 10^{2}

Figure 2

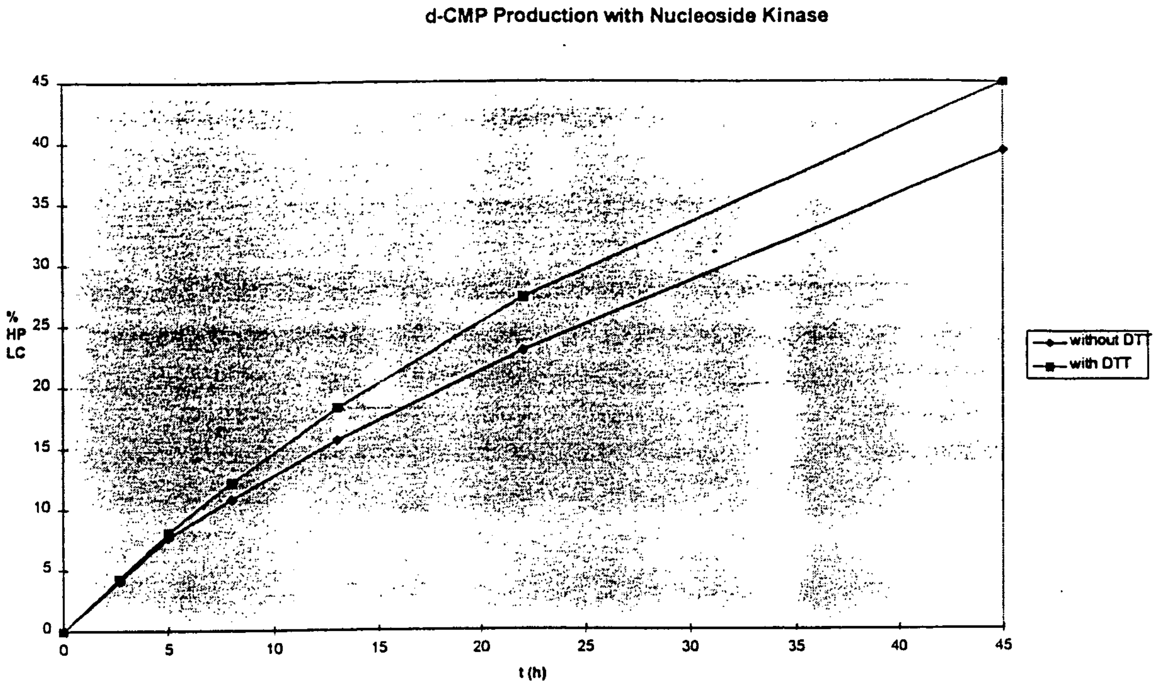

d-CMP Production with Nucleoside Kinase
45
40
35
30
25
20
15
10
5
0
% HPLC
0
5
10
15
20
25
30
35
40
45
t (h)
without DTT
with DTT

Figure 3

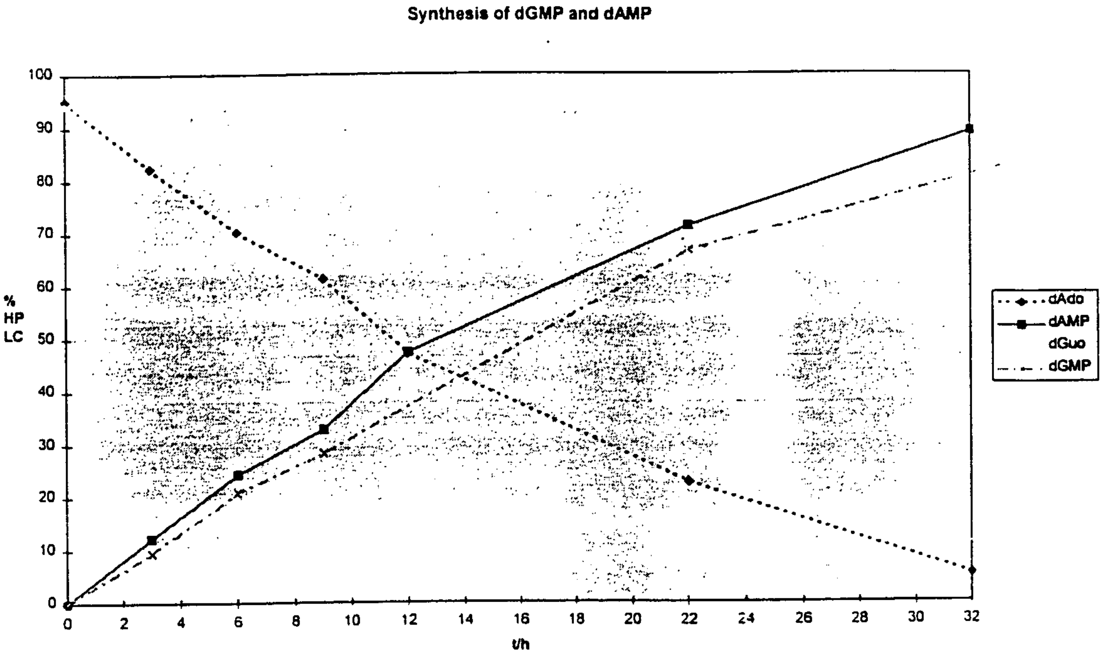

Synthesis of dGMP and dAMP
% HP LC
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
t/h
dAdo
dAMP
dGuo
dGMP

Figure 4

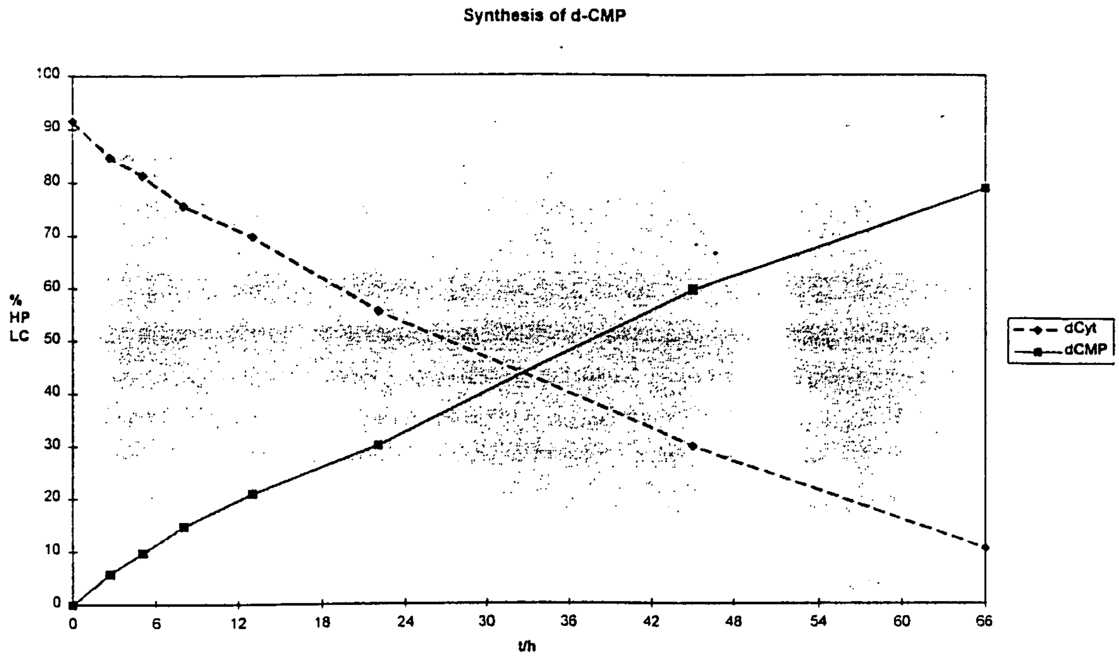

Synthesis of d-CMP
% HPLC
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
54
60
66
t/h
dCyt
dCMP

Figure 5

Seq. ID No.: 1

ATG GCG GAG GCA GCA TCC TGT GCC CGA AAG GGG ACC AAG TAC GCC

GAG GGC ACC CAG CCC TTC ACC GTC CTC ATC GAG GGC AAC ATC GGC

AGC GGG

AAG ACC ACG TAT TTG AAC CAC TTC GAG AAG TAC AAG AAC GAC ATT

TGC CTG

CTG ACC GAG CCC GTC GAG AAG TGG CGC AAC GTC AAC GGG GTA AAT

CTG CTG

GAG CTG ATG TAC AAA GAT CCC AAG AAG TGG GCC ATG CCC TTT CAG

AGT TAT

GTC ACG CTG ACC ATG CTG CAG TCG CAC ACC GCC CCA ACC AAC AAG

AAG CTA

AAA ATA ARG GAG CGC TCC ATT TTT AGC GCT CGC TAT TGC TTC GTG

GAG AAC

ATG CGA CGA AAC GGC TCG CTG GAG CAG GGC ATG TAC AAT ACG CTG

GAG GAG

TGG TAC AAG TTC ATC GAA GAG TCC ATT CAC CTG CAG GCG GAC CTC

ATC ATA

TAT CTG CGC ACC TCG CCG GAG GTG GCG TAC GAA CGC ATC CGG CAG

CGG GCT

CGT TCT GAG GAG AGC TGC GTG CCG CTT AAG TAC CTT CAG GAG CTG

CAT GAG

TTG CAC CAG GAC TGG TTG ATA CAC CAG AGA CGA CCG CAG TCG TGC

AAG GTC

CTA GTC CTC GAT GCC GAT CTG AAC CTG GAA AAC ATT GGC ACC GAG

TAC CAG

CGC TCG GAG AGC AGC ATA TTC GAC GCC ATC TCA AGT AAC CAA CAG

CCC TCG

CCG GTT CGT GTG TCG CCC AGC AAG CGC CAG AGG GTC GCC AGA **TAA**

Figure 6

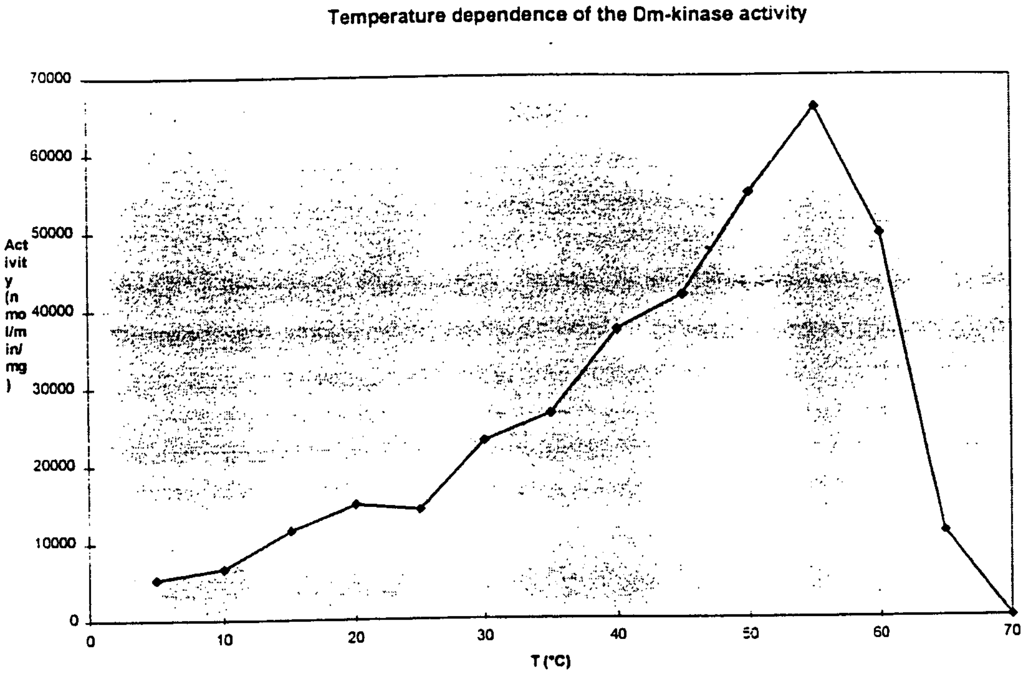

Temperature dependence of the Dm-kinase activity
Activity (nmol/min/mg)
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
70
T (°C)

Figure 7A

Stability of recombinant DM-kinase compared to native DM-kinase

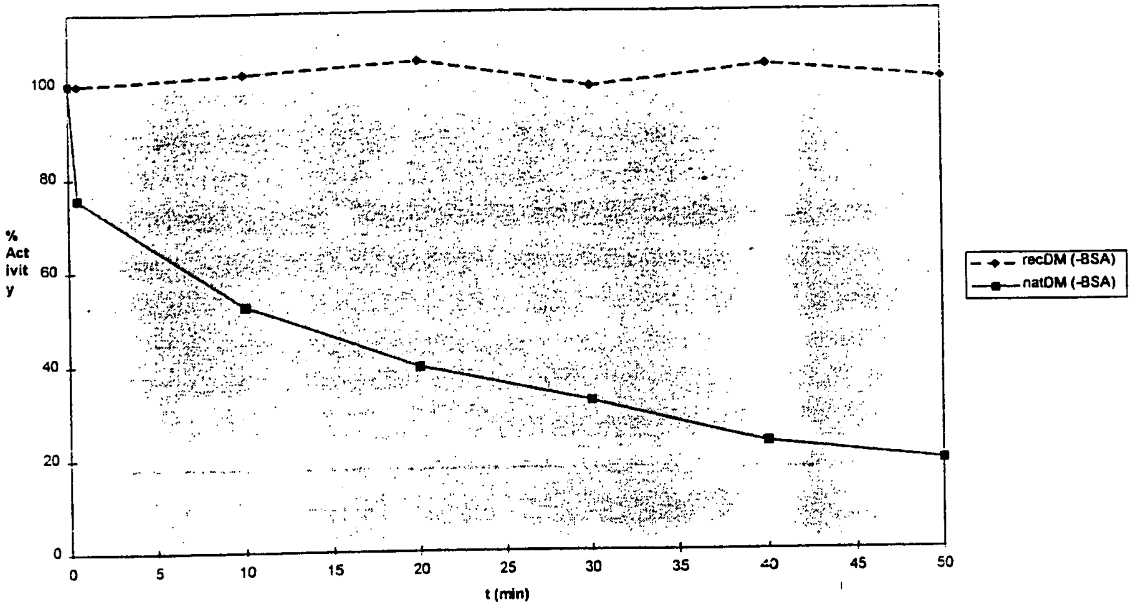
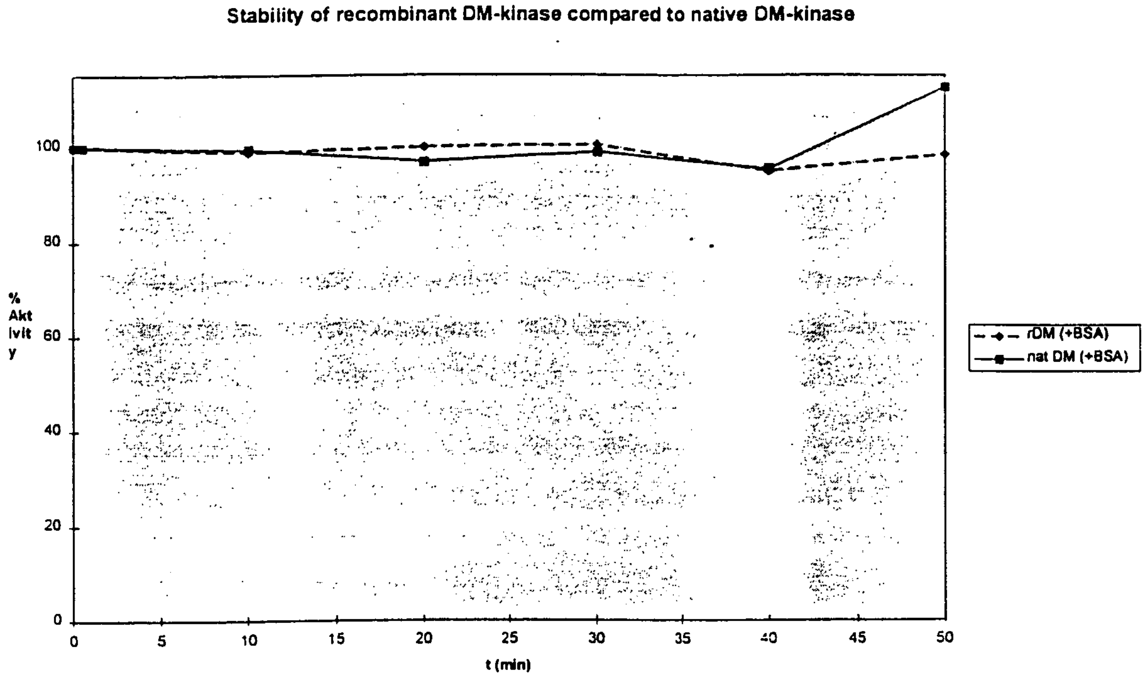


Figure 7B


Stability of recombinant DM-kinase compared to native DM-kinase